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...avioral and EEG Changes in Cats Induced by Prolonged Stimulation of Amygdala[1]

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ABSTRACT

...ONSO-DEFLORIDA, FRANCISCO AND JOSÉ M. R. DELGADO. *Lasting be...ral and EEG changes in cats induced by prolonged stimulation of amygdala.* Am. J. Physiol. 193(1): 223–229. 1958.—Social behavior of a colony of eight cats was recorded by time-lapse photography. A picture was taken every 9 seconds for 10 hours daily. Direct observations were also made. Films were analyzed, noting the time spent by each animal in selected patterns of behavior. Permanent cerebral electrodes were implanted in 15 cats and different areas stimulated for half a second every 5 seconds for 1 hour daily for 1–15 days. This type of prolonged stimulation of amygdala increased playful and contactual activities, modified aggressive and sexual behavior and produced other somatic, autonomic and behavioral effects. Local electrical activity was also influenced and seizure-like patterns were persistently recorded. Both behavioral and EEG phenomena were reversible (with 2 exceptions) and reproducible. Similar prolonged stimulation of other cerebral structures (internal capsule, falx cerebri and anterior hypothalamus) did not produce significant results.

THE ROLE of amygdaloid nuclei in behavior, particularly in emotional and sexual manifestations, has been repeatedly described in the literature (1–16). Some authors (17, 18) have used implanted electrodes to stimulate the amygdala in unanesthetized animals. The evoked effects included somatic and autonomic responses, oral activities, vocalization and well organized patterns of offensive and defensive behavior which were observed during or immediately after electrical stimulation of the brain applied for a few seconds. Lasting increase of food intake induced by electrical stimulation of the lateral hypothalamus for 1 hour daily has been reported by one of us (19). In the present study an attempt was made to learn whether or not lasting behavioral and/or electroencephalographic changes might be obtained by prolonged stimulation of the amygdaloid complex. Correlation between behavior and EEG was also analyzed. The amygdala was selected because it is concerned with the integration of patterns of emotional behavior and because of its possible role in epileptic manifestations. The use of a colony of animals permitted study of social relations. For this purpose, in addition to conventional methods, a technique was developed for time-lapse photographic recordings, ulterior quantifications and statistical analysis of selected categories of behavior.

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METHODS

Stimulation. Multilead needle electrodes were permanently implanted within the brain of 15 male cats. The electrodes were designed, placed, and the position of each lead determined according to the technique described by Delgado (20, 21). Bipolar stimulation was applied with square bidirectional waves of 0.2 msec. pulse duration, 100 cps, intensity from 0.2 to 4.0 ma for 0.5 second every 5 seconds,

during 1 hour daily for 1–15 days. Points with low convulsive threshold were selected for stimulation. Intensity was adjusted below this threshold to evoke only slight motor effects.

EEG Recordings were obtained with a Grass electroencephalograph from the unanesthetized, unrestrained animals which were usually tractable; only occasional movement artefacts interfered with the recordings.

Records of Behavior. The cats were grouped in a glass-fronted cage, $7\frac{1}{2} \times 3 \times 3\frac{1}{2}$ feet, males and females in ratios of 5:3, 3:1 or 2:1; sometimes two small groups were studied simultaneously by using a partition in the cage. Each group had one implanted electrode cat (IEC). A time-lapse camera took one picture every 9 seconds for 10 hours each day (usually from 8:30 A.M. to 6:30 P.M.). Temperature was kept at about 20°C; illumination was furnished by 24 fluorescent lights (20 w, white) with a lightmeter reading of 13. The room was sound proof. Diet was the 'synthetic' type of Krehl *et al.* (22) slightly modified.[3] Films were analyzed according to different patterns of behavior selected after preliminary study: *1)* walking or standing on all fours. *2)* Aggressiveness, evidenced by threatening, snarling, flattening the ears, piloerection, fighting position and striking. Aggressiveness was usually defensive, muscle tonus seemed increased, the forelegs were semiflexed and the cat watched its opponent intently. *3)* Playful activity, shown by gentle pawing of objects or by a pretense to fight. Also included in this category was a quite characteristic pattern that appeared clearly in the time-lapse films: the animal lay on one flank with fore and hindlegs extended, the body relaxed and generally motionless, but it gently pawed (1–3 times in 7 min.) any object or a passing cat. *4)* Contactual activity, shown by sniffing, licking, nuzzling and rubbing. This pattern may be regarded as sexual activity; it was not only performed between members of the different sexes as sexual play or courtship but was often present as well between males, although it was seldom seen between anestrus females. Some of the patterns could not be differentiated from purely investigatory activity which might have no connection at all, however, with sexual activity. Categories were usually easily identifiable in the films, but borderline patterns were sometimes difficult to appraise and more frequent pictures or additional direct observations might be necessary.

Direct observations and 8–15 days of recordings were made for each IEC before, during, and in some animals, after stimulation. Some cats were recorded daily and others every other day, with EEGs made on alternate days. IEC was removed from the cage for 1 hour for control or stimulation during each 10-hour record. This period was chosen at random for some animals and at a fixed hour for others, during the first 4 hours of the 10-hour recording. The timing and arrangement of colonies varied for different IECs but conditions were always as similar as possible during control and stimulation for each one.

Location of Electrodes. After the study of each IEC was completed the animal was killed under anesthesia and perfused with formalin 10%; paraffin sections of the brain were made and stained by the Kluever method (23). The needle tracts were microscopically examined, and since there was no histological evidence of the position of each contact within the tract, position of each point was calculated by comparison with the preserved array of electrodes. The anatomical structures involved included both cortical medial and basolateral part of the amygdala, anterior hypothalamus, internal capsule, anterior hippocampus and falx cerebri.

RESULTS

Observations During the 1-Hour Stimulation Period. Several somatomotor and autonomic responses were found as the result of prolonged intermittent stimulation of the amygdaloid nucleus. The most common was ipsilateral involvement of facial muscles synchronous with the stimulation bursts: closing the eye, lowering the ear, retraction of mouth and rhythmic movements of upper lip and nostrils as in sniffing. Licking the chops was a more integrated pattern often observed and highly integrated patterns also occasionally developed, such as smelling the floor and walls of the observation box, which were associated with flexing and other exploratory movements.

[3] We are indebted to Dr. J. J. Barboriak from the Dept. of Biochemistry, Yale University Nutrition Laboratory, for assistance in this matter.

Several times cats suddenly shifted from a quiet attitude to very active playfulness, pawing any object available.

Autonomic effects consisted in pupil dilatation and piloerection; somatomotor effects, in general, decreased in intensity during the hour stimulation. Afterdischarges in most cats appeared as rhythmic blinking, opening and closing the mouth with retraction of the corners, propulsion of the tongue, plentiful salivation, dilatation of pupils and cessation of motor activity. Sometimes, however, the animal moved around or compulsively tried to escape. Usually afterdischarges stopped spontaneously regardless of accompanying intermittent bursts. They generally lasted from 3 seconds to more than a minute, but sometimes lasted up to 10 minutes, at which point the stimulator was turned off until the epileptic crisis disappeared. Afterdischarges began at unpredictable times and were observed regardless of the fact that in all animals intensity of current was below convulsive threshold.

Qualitative Observations During Stimulation Days. Intermittent electrical stimulation of amygdala for 1 hour produced in general an increase of time spent in some activities and determined the appearance of afterdischarges and automatisms. Some stimulated cats persistently tried to cover feces excreted by other cats, which was never observed in nonstimulated animals. Some less tractable animals became gentler after amygdala activation, except for crises of compulsive behavior; in such animals rough handling or administration of noxious stimuli usually failed to elicit the previously observed anger reactions.

Several times male cats were observed crouching like estrus females but raising of the pelvis, turning the tail to one side and treading were not so conspicuous as in the female. Occasionally the electrically stimulated males were clamped by other males and two animals were repeatedly mounted, although homosexual mounting was most uncommon among normal cats in our experiments.

Motor afterdischarges, like those of the stimulation period, were frequently seen immediately and/or several minutes after stimulation was stopped. Occasionally afterdischarges were observed at intervals during the day or for several days after the stimulation period. The seizures developed a few hours after stimulation, were shorter in duration and were discrete: slight blinking, dilatation of pupils and movements of nostrils and upper lip with no salivation. Occasionally catatonic stances occurred, lasting as long as 35 seconds. When the animal was returned to the colony with afterdischarges still present, it was examined and sniffed thoroughly by the other cats. The stimulated cat usually did not respond in any way but remained standing or seated, moving its head slowly from one side to the other, obviously disoriented. Occasionally animals in this state walked back and forth through the colony, sometimes provoking rage among the other cats and being struck at without responding. At other times the stimulated cat would attack without good orientation. Outside the colony the animal in this state showed compulsive behavior; an apparently and otherwise peaceful cat might jump off the observation table or struggle

TABLE 1. INCREMENTS AND DECREMENTS FOR TIME SPENT IN PATTERNS OF BEHAVIOR*

Cat No.	Wk	Agr	Ct	Pl
	Amygdala Stimulation			
1	−38.3	2.2	1.3	72.0
3	*42.0*	*−4.8*	*31.0*	57.7
18	−72.6	−6.5	−0.5	50.8
19	3.6	−0.2	0.0	10.8
28	8.9	0.2	0.4	118.3
37	18.2	0.0	*31.8*	97.5
	Stimulation in Other Structures†			
2	−36.5	−11.6	−1.2	−5.7
4	31.3	0.2	1.3	2.6
14	13.8	6.0	−0.7	−6.8
15	54.2	−6.5	−2.3	−5.1
16	−17.7	0.3	0.1	*12.6*
17	8.2	*10.0*	0.4	−3.6
32	10.2	0.0	2.8	13.4

* Increments and decrements were obtained by subtracting the mean for the control period from the corresponding mean of the stimulation period. Significance was calculated by ranking procedures (24, 25) and $P < 0.05$ is indicated in italics.

† Other stimulated structures were internal capsule (*cats 2 and 32*); falx cerebri (*cat 4*); anterior hypothalamus (*cats 15, 16 and 17*).

showed reversible effects for playful activity, thus allowing for a second trial which, however, elicited the fatal epileptic crisis described. No other cats were studied in connection with reversibility of quantified behavioral changes.

Electroencephalographic Activity. Spontaneous electrical activity in unanesthetized animals shows patterns which can be repeated with similar characteristics on different days (21). An example is given in figure 1*A*, where activity of pyriform cortex, basolateral amygdala, globus pallidus and putamen was recorded in both sides of the brain. Regular fast activity with amplitude about 50 μv mixed with some slower frequencies was often observed in amygdala.

Intermittent electrical stimulation of amygdala for one hour as described in METHODS evoked lasting changes in the electrical activity of the brain. A few minutes after the 1-hour period of bipolar stimulation the activity appeared rather disorganized in both sides, with slow swings of frequency about 1–2/sec. and amplitude of 300 μv, more prominent in the stimulated side (fig. 1*b*). A typical feature was the appearance in the stimulated points of repeated bursts of about 25 cps above 100 μv, each lasting for 200–250 msec. This effect lasted a few hours in some animals and up to 3 days in others. One day after stimulation isolated but frequent (e.g., 4–10/sec.) spikes or spikes and waves alternated with repetitive spiking (2–5/sec.) and with short bursts of high voltage spiky activity, as shown in figure 1*C*. Sometimes a burst of spikes lasting several seconds was evoked by auditory stimulation such as clapping hands. Contralateral to the stimulation electrical activity was depressed and propagation of spikes was rarely observed. During the recordings in figure 1*C* the cat did not seem disturbed, no automatisms were present and the animal behaved as usual. However, at other times motor afterdischarges were observed. In several cats, such as *cat 28*, it was possible to record the cerebral electrical activity during motor (face) and autonomic (salivation, pupillary dilatation) afterdischarges and one example is given in figure 2, where the described effects appeared at the arrow, when a well organized spike and wave activity was recorded in all leads. In other animals automatisms were concomitant with high voltage (about 1 mv) spikes and waves

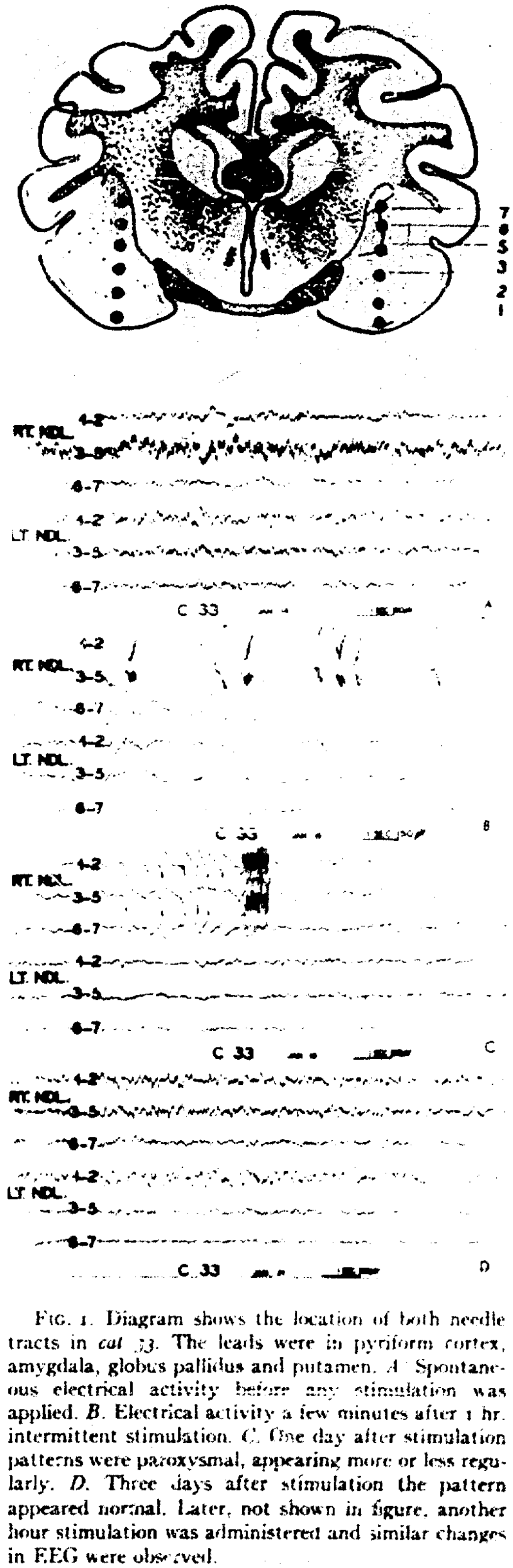

FIG. 1. Diagram shows the location of both needle tracts in *cat 33*. The leads were in pyriform cortex, amygdala, globus pallidus and putamen. *A*. Spontaneous electrical activity before any stimulation was applied. *B*. Electrical activity a few minutes after 1 hr. intermittent stimulation. *C*. One day after stimulation patterns were paroxysmal, appearing more or less regularly. *D*. Three days after stimulation the pattern appeared normal. Later, not shown in figure, another hour stimulation was administered and similar changes in EEG were observed.

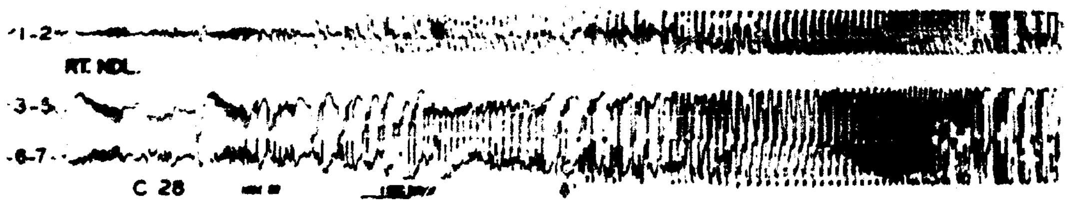

FIG. 2. Development of spike and wave activity in *cat 28* during the 3rd day of stimulation. At the *arrow* motor and autonomic manifestations appeared. The high frequency bursts before the *arrow* appeared at variable times without motor and autonomic manifestations or automatisms.

which were repeated about once every 2 seconds. At variable times, usually 4–8 days after stimulation, afterdischarges disappeared, as shown in figure 1*D*. However, electrical activity could have some differences as compared with the control period, as is shown in this figure by the slowing down in linkages LT NDL 1 2.

Pathology. Microscopic study of the brain revealed a destruction along the needle tract without neural elements but with polymorphonuclear cells, capillary proliferation and collagen tissue. Around the tract there was a rather delimited capsule with abundant fibroblasts, collagen tissue and some neuroglia and microglia cells. The size of the tract varied, the usual diameter being about 1 mm. Beyond the capsule neurons appeared well preserved in general. Histological study of the points which were repeatedly electrically stimulated did not reveal any special characteristics, nor could any difference be observed between stimulated and nonstimulated sides of the brain. Needle tracts of two animals which, as a result of stimulation had continued seizure activity, were histologically similar to the rest of the group. In effect, in our experiments prolonged electrical stimulation of some points of the brain did not produce observable histological changes.

DISCUSSION

In agreement with previous investigations (8, 17, 18, 26) a variety of somatic, autonomic and behavioral effects were evoked in our studies of electrical stimulation of the amygdala. In the superior points of this region close to putamen effects were usually contralateral, while in lower structures the effects were more often ipsilateral. In some cats points in the amygdala close to the pyriform cortex failed to evoke the ipsilateral movements observed by stimulation of more superior structures. This argues against a spread of current to cranial nerves which would cause ipsilateral effects, as does the fact that some of the motor afterdischarges, present after cessation of stimulation, were ipsilateral.

It should be emphasized that motor afterdischarges result from prolonged stimulation using intensities below seizure threshold. This indicates a long temporal summation at the initiation of the localized motor seizures because the half second of stimulation was spaced every $4\frac{1}{2}$ seconds, as explained in METHODS. The afterdischarges usually stopped in spite of the persistence of the intermittent stimulation, which indicates a limiting factor in the temporal summation and the independence of the mechanism responsible for the termination of the afterdischarges.

As shown by direct observation, prolonged stimulation of the amygdaloid nucleus could increase or decrease the aggressive behavior of the same cat; it would seem, therefore, that positive and negative influences upon aggressiveness exist in different points of the amygdaloid complex, a possibility that would explain some of the contradictory results in the literature (2, 9) and needs further experimental confirmation.

Recording of behavior by time-lapse photography and its quantification by counting the number of pictures in which a particular category of behavior is present offers the advantage of objectivity of results, the possibility of statistical treatment of figures and allows study of various types of behavior simultaneously in several animals. One limitation of the method is that some categories are difficult to identify in the films. Play, for example, may be confused with fighting and more frequent pictures, or additional direct observations sometimes are necessary. The time spent in analysis depends upon the aim of the investigator. An 8-hour film may be scanned in a few minutes to learn the relationship between sleep and wakefulness, but it takes many

hours to assemble detailed data on behavior. A critical study of the method will be made elsewhere (27); in the present paper we present its application to the study of the effects of amygdala stimulation upon group behavior. Increase in playful behavior was statistically significant during the days of brain stimulation; it was reversible and could be duplicated. It should be remembered that photographic recordings were always made of the whole colony and the IEC was removed from the group for brain stimulation, so that we were not recording the direct results of cerebral stimulation but only the effects which outlasted the stimulation period. Play is a supposedly highly integrated type of behavior; its pattern, as shown by the study of films and in supplementary direct observation of the colony, did not appear to be modified by amygdala stimulation, but the time spent in this activity was increased. Modification of other types of behavior was less clear, but contactual activity also seemed to be increased by stimulation of the amygdala.

In our experiments qualitative and quantitative modifications of behavior showed correlation with local disturbances of electrical activity of the amygdaloid region. Behavioral and electroencephalographic changes lasted for hours or days after the stimulation period; with the two exceptions noted, both were reversible and could be duplicated. Compulsive behavior developed occasionally and in general the modification of behavior was only quantitative, with social activities appearing qualitatively normal. Our results agree with the correlation between seizure activity and behavioral somatic and autonomic changes evoked by stimulation of the amygdaloid complex as described by Naquet in Gastaut's laboratories (28).

The potential danger of electrical stimulation of amygdala and the epileptogenic activity of the area were demonstrated by the two cats in which the evoked seizures lasted for hours in one case and for 27 days in the other, and which were probably the cause of death.

That the determining factor of the effects observed was not the injury produced by the electrodes but the electrical stimulation was demonstrated by the following facts: *a*) electrical stimulation was sine qua non in altering EEG and behavior; *b*) effects were reversible with exceptions and reproducible; *c*) it was not possible to establish any correlation between the degree of the effects and extent of the histological reaction; *d*) there were no histological differences between stimulated and nonstimulated points, or between stimulated and nonstimulated cats which died in epileptic status. Whether or not injury cooperated in the development of the effects cannot be decided without further investigation.

REFERENCES

1. ANAND, B. W. AND J. R. BROBECK. *J. Neurophysiol.* 15: 421, 1952.
2. BARD, P. AND V. B. MOUNTCASTLE. *Res. Publ. A. Nerv. & Ment. Dis.* 27: 262, 1948.
3. BRADY, J. R., L. SCHREINER, I. GELLER AND A. KLING. *J. Comp. & Physiol. Psychol.* 47: 179, 1954.
4. DELL, P. *J. physiol., Paris* 44: 471, 1952.
5. FULTON, J. F. *Frontal Lobotomy and Affective Behavior.* New York: Norton, 1951.
6. GASTAUT, H. *J. physiol., Paris* 44: 431, 1952.
7. GASTAUT, H., R. NAQUET AND A. ROGER. *Rev. neurol.* 87: 224, 1952.
8. KAADA, B. R. *Acta physiol. scandinav.* 24 (suppl. 83), 285 pp., 1951.
9. KLUEVER, H. AND P. C. BUCY. *Am. J. Physiol.* 119: 352, 1937.
10. KLUEVER, H. AND P. C. BUCY. *J. Psychol.* 5: 33, 1938.
11. KLUEVER, H. AND P. C. BUCY. *Arch. Neurol. & Psychiat.* 42: 979, 1939.
12. MACLEAN, P. D. *J. Neurosurg.* 11: 29, 1954.
13. PAPEZ, J. W. *Arch. Neurol. & Psychiat.* 38: 725, 1937.
14. PRIBRAM, K. H. AND L. KRUGER. *Ann. New York Acad. Sc.* 58: 109, 1954.
15. SCHREINER, L. AND A. KLING. *J. Neurophysiol.* 16: 643, 1953.
16. SPIEGEL, E. A., H. R. MILLER AND M. J. OPPENHEIMER. *J. Neurophysiol.* 3: 538, 1940.
17. GASTAUT, H., R. VIGOUROUX, J. CORRIOL AND M. BADIER. *J. physiol., Paris* 43: 740, 1951.
18. GASTAUT, H., R. NAQUET, R. VIGOUROUX AND J. CORRIOL. *Rev. neurol.* 8[illegible]: 310, 1952.
19. DELGADO, J. M. R. AND B. W. ANAND. *Am. J. Physiol.* 172: 162, 1953.
20. DELGADO, J. M. R. *Yale J. Biol. & Med.* 24: 351, 1952.
21. DELGADO, J. M. R. *Electroencephalog. & Clin. Neurophysiol.* 7: 637, 1955.
22. KREHL, W. A., G. R. COWGILL AND A. D. WHEDON. *J. Nutrition* [illegible], 1955.
23. KLUEVER, H. AND E. BARRERA. *J. exper. Neurol.* 12: 400, 1953.
24. WHITE, C. *Biometrics* [illegible], 1952.
25. WILCOXON, F. *Biometric Bull.* 1: 80, 1945.
26. MACLEAN, P. AND J. M. R. DELGADO. *Electroencephalog. & Clin. Neurophysiol.* 5: 91, 1953.
27. DELGADO, J. M. R. AND K. RODRIGUEZ DELGADO. XV Internat. Congress Psychol., Brussels, 1957. In press.
28. NAQUET, R. *Sur les Fonctions du Rhinencéphale D'après les Résultats de la Stimulation Chez le Chat* (Thèse, Marseille), [illegible].

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